The Effect of Prostaglandin D_2 on the Response of Human Skin to Histamine*

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The interaction of prostaglandin D_2 (PGD_2) and histamine in human skin was studied by intradermal injection of the compounds alone or in combination in healthy volunteers. Responses were recorded by measurement of areas of wheal and erythema, and changes in cutaneous blood flow quantified using a laser Doppler flow meter. The effect of a near-threshold dose of PGD_2 on histamine dose-response relationships and on the response to a single low dose of histamine were examined. Histamine caused dose-related increases in blood flow and in areas of wheal and erythema in human skin. Prostaglandin D_2 caused dose-related increases in blood flow and erythema area, but not wheal area, in the dose range used. When the compounds were injected together, PGD_2 did not potentiate the increase in blood flow and areas of wheal and erythema due to histamine. The modest augmentation of histamine response in the presence of PGD_2 could be attributed to summation alone. The role of PGD_2 in cutaneous disorders such as the physical urticarias, in which its release has been demonstrated, is therefore uncertain. In the amounts measured in the urticarias, it is unlikely alone to cause a significant cutaneous response; nor does it appear to act by potentiation of the response to histamine. *J Invest Dermatol 89: 245–248, 1987*

Intradermal injection of prostaglandin D_2 (PGD_2) causes erythema and whealing in human skin, although it is approximately 5 times less potent than prostaglandin E_2 [1,2]. PGD_2 is the major cyclooxygenase product of the human mast cell and is released in parallel with histamine by passively sensitized human skin in vitro [3] and by human pulmonary mast cells incubated with anti-IgE [4,5]. Prostaglandin D_2 generation has also been demonstrated in vivo in immediate allergic reactions in human skin [6]. Recent work has demonstrated concurrent release of immunoreactive PGD_2 and histamine into the venous blood draining cold-challenged arms in patients with cold urticaria, although the peak histamine concentration was between 271- and 1242-fold greater than the peak concentration of PGD_2 on a molar basis [7]. Studies on patients with heat urticaria have yielded similar results [8]. PGD_2 released in the amounts measured is unlikely to contribute directly to the cutaneous changes in urticaria, but it may act synergistically with histamine. Indeed, PGD_2 has been shown to potentiate the increase in vascular permeability induced by histamine in rat skin [1].

We have therefore investigated the interactions between PGD_2 and histamine in human skin by measuring wheal and erythema areas and cutaneous blood flow, following intradermal injection.

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Abbreviation:

PGD_2: prostaglandin D_2

MATERIALS AND METHODS

Subjects Nine healthy volunteers (6 men, 3 women, age range 25–73), none of whom was taking any medication, gave informed consent to participate in the study, which was approved by the Research Subcommittee of St. John's Hospital.

Intradermal Injections Stock solutions of PGD_2 (60 μM, Sigma Chemical Co., St. Louis, Missouri) and histamine dihydrochloride (180 μM, Sigma) were prepared in phosphate-buffered saline (final pH 7.2) and stored at -70°C. On the day of the experiment, appropriate dilutions were made in phosphate-buffered saline and sterilized by filtration (millipore, Millex-GS 0.22 μM) immediately prior to use. Fifty microliter volumes of histamine, PGD_2, or a mixture of the 2 compounds were injected intradermally into the back using a 25-gauge needle, allocating the injections randomly to different sites in order to reduce any bias from spatial variability in cutaneous response [9]. All experiments were carried out at the same time of day, at an ambient temperature of 23°C.

Measurement of Areas of Wheal and Erythema Areas of wheal and erythema were measured at various time intervals after injection by tracing the outlines onto a transparent polythene sheet. The sheet was photocopied and the wheal and flare images cut out and weighed. From this, the areas could be calculated.

Measurement of Cutaneous Blood Flow A laser Doppler flow meter (Perimed II, Periflux, Sweden) was used for the quantitative measurement of changes in microcirculatory flow induced by histamine and PGD_2. This noninvasive optical technique has been found to be a valuable and accurate method in the study of cutaneous inflammation [10–12]. Preliminary experiments showed that readings were variable when the probe was placed over the wheal; however, measurements taken within the flare but adjacent to the wheal gave reproducible results that correlated well with a visual assessment of the degree of erythema. All measurements were therefore made with the probe immediately adjacent to the wheal, as recommended by Serup and Staberg [12]. The probe

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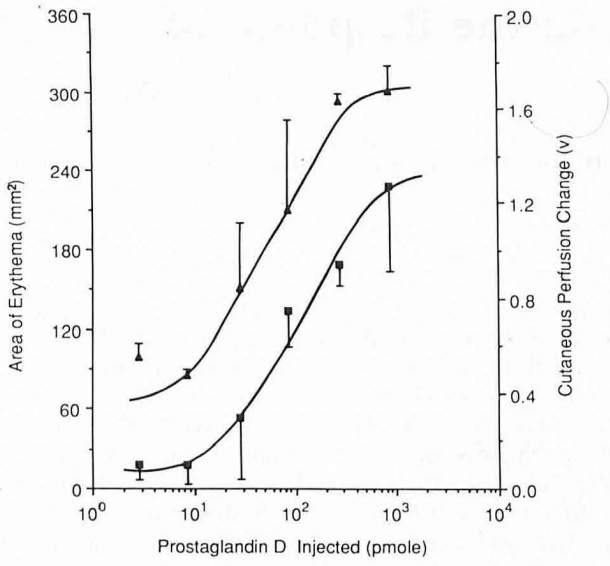

Figure 1. Dose-response curves for area of erythema and cutaneous blood flow changes induced by intradermal injection of prostaglandin D_2 in healthy volunteers. Erythema area (*triangles*) was measured 10 min after injection. Cutaneous blood flow (*squares*) was measured 30 min after injection. Each point represents the mean ± SEM; n = 3.

was held in position for 2.5 min at each site, with the use of a perspex holder taped to the skin. This period of application produced a stable blood flow reading at each site and the mean value (V) over the 2.5 min period was then recorded. Readings were also taken from normal skin at 6 different sites on the back and the mean of these values subtracted from the reading obtained at injected sites, to give the increment in cutaneous blood flow induced by the injection.

PGD_2 Dose-Response Curves Dose-response relationships for PGD_2 alone were established by injecting doses of 3, 9, 28, 85, 284, and 851 pmol in each of 3 subjects and measuring the responses at 10 min (areas of wheal and erythema) and 30 min (cutaneous blood flow). In the remaining 6 subjects, the erythema threshold of PGD_2 was determined without establishing a complete dose-response relationship. For the purposes of this study, the dose of PGD_2 referred to as the threshold was that which elicited an erythema that was confined to the area of the wheal.

Histamine Dose-Response Curves in the Presence and Absence of a Near-Threshold Dose of PGD_2 Dose-response relationships for histamine alone were established by injecting doses of 27, 90, 270, and 900 pmol, and 2.7 and 9 nmol in each of 7 subjects. Areas of erythema were measured in all 7 subjects; cutaneous blood flow and wheal areas were measured in five. Laser Doppler measurements were carried out at 5 min; areas of wheal and erythema were measured at 10 min. In each volunteer, the same doses of histamine were then injected in the presence of a dose of PGD_2 at or a little above the threshold dose, and the measurements repeated.

Effects of a Near-Threshold Dose of PGD_2 on the Response to a Single Dose of Histamine (90 pmol/site) The effects of a dose of PGD_2 at or a little above the threshold dose on the response to a single low dose of histamine (90 pmol/site) were examined in 6 subjects. For this experiment, each injection of histamine alone and histamine with PGD_2 was done in triplicate. Areas of wheal and erythema were measured at 3, 6, and 15 min; cutaneous blood flow was measured at 5 (7 subjects), 8, and 13 min.

RESULTS

PGD_2 Dose-Response Curves Intradermal injections of PGD_2 alone, in amounts ranging from 3–851 pmol per site, caused dose-related erythema. The erythema was darker than that induced by histamine and was of longer duration, attaining a maximum at 15–30 min and disappearing by 5 h. An erythema threshold dose for PGD_2 in each subject was established and found to vary from 3–85 pmol. The dose-response curve obtained for PGD_2-induced erythema is shown in Fig 1. In the amounts used, PGD_2 only elicited a minor degree of whealing, the area of which was not dose-related. However, a dose-response relationship was found for cutaneous blood flow (Fig 1).

There was a large intersubject variation in the responses to both PGD_2 and histamine, accounting for the large standard errors obtained.

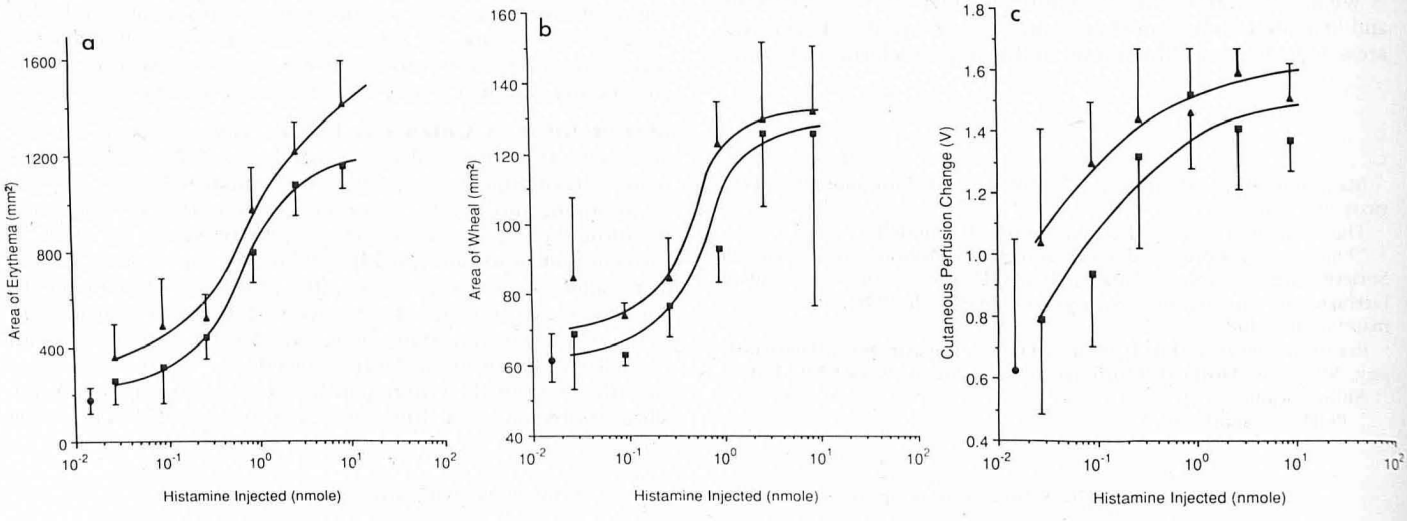

Figure 2. Dose-response curve for erythema area, wheal area, and cutaneous blood flow changes induced by intradermal injection of histamine alone and with a near-threshold dose of prostaglandin D_2, in healthy volunteers. *Square* = histamine alone; *circle* = prostaglandin D_2 alone; *triangle* = histamine + prostaglandin D_2. Each point represents the mean ± SEM. *a,* Area of erythema, measured 10 min after injection; n = 7. *b,* Area of wheal, measured 10 min after injection; n = 5. *c,* Cutaneous blood flow change, measured 5 min after injection; n = 5.

Histamine Dose-Response Curves in the Presence and Absence of a Near-Threshold Dose of PGD_2 Intradermal injections of histamine, in quantities ranging from 27 pmol to 9 nmol, caused dose-related erythema, whealing, and increases in cutaneous blood flow. The erythema was maximal at 5 min and had disappeared by 1 h. Whealing disappeared within 3 h. The dose-response curves for cutaneous blood flow were variable from subject to subject; in some cases there was a near-linear relationship between blood flow and histamine dose over the dose range examined, whereas in others the lowest dose of histamine used elicited near-maximal blood flow with little further increase at higher doses.

The effects of a near-threshold dose of PGD_2 on histamine-induced erythema was investigated in 7 separate experiments and on histamine-induced whealing and cutaneous blood flow in 5 experiments. Prostaglandin D_2 consistently caused a slight leftward displacement of the dose-response curves (Fig 2). This displacement appeared to be no greater than that which would be expected from summation of the individual responses to histamine and PGD_2.

Effect of a Near-Threshold Dose of PGD_2 on the Response to a Single Dose of Histamine (90 pmol/site) The results of these experiments are summarized in Tables I and II and have been expressed as mean ± SEM of values in all subjects at each time interval. At this concentration, histamine caused erythema, whealing, and increased blood flow. As can be seen from the Tables, the addition of PGD_2 produced only a modest increase in response. None of the differences was statistically significant except for erythema area measured at 15 min after injection ($p < 0.05$, Wilcoxon's test). The principal difference observed was that the responses to the histamine and PGD_2 combination were more purple in color and longer lasting than the responses to histamine alone. This effect explains the somewhat larger difference in erythema area between histamine with and without PGD_2 observed at 15 min, compared with the 3 and 6 min readings.

DISCUSSION

The results of this study suggest that, under the conditions used, PGD_2 does not potentiate the response of human skin to histamine, with respect to the size of the erythema and wheal, and cutaneous blood flow. The modest augmentation of responses in the presence of PGD_2 can be attributed to summation alone. These results confirm and extend those of a recent study by Barnes and Heavey [13], who examined the effect of PGD_2 on wheal response to intradermal histamine, and found no effect on wheal area or volume. Since Flower and associates [1] demonstrated potentiation of histamine-induced vascular permeability increases by PGD_2 in rat skin, it is likely that species differences exist in the cutaneous responses to these compounds.

The amounts of PGD_2 required to cause erythema and edema in this study are greater than those measured in blood during heat and cold urticaria. Thus PGD_2 is unlikely to contribute directly to the cutaneous changes observed in these conditions. Furthermore, since we have demonstrated that PGD_2 does not potentiate the vascular responses to histamine, which is released concurrently, it is unlikely that a synergistic interaction between the 2 compounds occurs with respect to increased permeability and erythema. The lack of a role of PGD_2 in heat and cold urticaria would also be supported by the therapeutic ineffectiveness of cyclooxygenase inhibitors, such as indomethacin, in these conditions (A. Kobza Black, personal communication).

Prostaglandin D_2 production by mast cells is believed to contribute substantially to symptoms such as flushing and hypotension in systemic mastocytosis [14], but its biologic role in skin disorders remains unclear. The possibility that the PGD_2 metabolite, 9α, 11β-prostaglandin F_2 [15] has a role in the mediation of cutaneous inflammation is currently under investigation.

Table I. Effect of a Near-Threshold Dose of Prostaglandin D_2 (PGD_2) on the Areas of Erythema and Wheal Induced by the Intradermal Injection of 90 pmol Histamine (H) in Healthy Volunteers

Time (min)	Number of Subjects	Reagents Injected	Wheal Area (mm^2)	Erythema Area (mm^2)
3	6	H	77 ± 9	735 ± 167
		H + PGD_2	77 ± 8	861 ± 143
6	6	H	78 ± 9	641 ± 184
		H + PGD_2	84 ± 10	756 ± 129
15	6	H	82 ± 10	467 ± 159
		H + PGD_2	86 ± 8	625 ± 182

Readings are made at 3–15 min intervals after injection. Values represent mean ± SEM.

Table II. Effect of a Near-Threshold Dose of Prostaglandin D_2 (PGD_2) on the Cutaneous Blood Flow Changes Induced by the Intradermal Injection of 90 pmol Histamine (H) in Healthy Volunteers

Time (min)	Number of Subjects	Reagents Injected	Cutaneous Blood Flow Change (V)
5	7	H	1.22 ± 0.15
		H + PGD_2	1.32 ± 0.15
8	6	H	1.32 ± 0.17
		H + PGD_2	1.32 ± 0.15
13	3	H	1.34 ± 0.13
		H + PGD_2	1.37 ± 0.08

Readings are made at 5–13 min intervals after injection. Values represent mean ± SEM.

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